

Chapter 21

Genomics of Quality Traits

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Abstract The quality attributes of cereal grains are valued in the context of a complex food chain that integrates outputs achievable by breeding, production, and processing. New processing technologies, environmental change, and changes in consumer preferences demand that quality attributes of wheat and barley need to be continually modified. The advances in the genomics of quality described in this chapter provide the basis for ensuring that the genetic approaches encompassing the complexities of the gene networks underpinning quality attributes can meet the challenges presented by the rapid changes occurring within the food chain.

21.1 Introduction

The wheat and barley industry is currently undergoing major changes as the value of the grain increases due to the combined pressures of limited world-stocks resulting from droughts and loss of available land, and competition for use of the grain for biofuels and feed. The marketers and growers of major crops such as wheat and barley need to increase their productivity by 10–20% over the next decade (utilizing a reduced growing area) while maintaining the quality attributes demanded by increasingly sophisticated customers. The market in the India-Asia region is growing most rapidly and wheat/barley quality improvement has a significant part to play in developing the linkages with customers in these markets. New advances in the engineering of large-scale processing equipment as well as changes in the market place resulting from the striking demographic changes in the region indicate that traditional quality attributes of wheat and barley will also need to evolve. Genome level studies are considered crucial to defining the genes and gene networks underpinning the functional qualities of flour and malt for a range of end products and health claims.

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The grain of the cereal crops provides a large portion of both the total calories and protein in the human diet. Many of the chromosome regions affecting quality attributes are now amenable to analysis at the genomic structure/function level and in this chapter the aspects contributing to wheat and barley quality attributes targeted to specific end products are discussed. Although we have described some recent work on barley cell wall structure and the implications for this work on human health, the broader aspects of nutrition for wheat and barley are not within the scope of this chapter.

21.2 Genomics of Barley Quality

Barley grain is used for human food, animal feed and for the production of malt. Malt finds its way into many food products but the most important are beer and whiskey.

Animal feed qualities for barley are of interest as the cost of grain increases due to competition from the biofuels area. Although the emphasis for feed use is on total yield, the requirements for monogastric animals (pigs and poultry) are quite different from those for ruminants (feedlot and dairy), (Gali et al. 1998; White et al. 2007). Pigs and poultry demand a high available energy intake, with easily digestible starch, while for ruminants the industry requires grains that are slower fermenting to avoid acidosis. Grain as a source of carbohydrate is a key focus.

Traditionally, feed barley has been spring or winter type and six rowed and was selected primarily for yield while malting barley has been exclusively spring type and in most cases two rowed. Wild barley (*Hordeum vulgare* ssp *spontaneum*) is also two rowed but a mutation at a single gene *Vrs1* led to the six row forms which were generally higher yielding. A positional cloning approach was recently used to isolate the *Vrs1* gene (Komatsuda et al. 2007). The six rowed phenotype resulted from loss of function of *Vrs1* and conversion of rudimentary lateral spikelets into fully functional spikelets.

A second gene that has been important for both feed and human food barley is the hulless locus (*nud*) on 7H. In hulless lines the glumes detach from the pericarp during threshing to produce a hulless or naked grain. Several countries have now established breeding programs focused on producing hulless or naked barley specifically for the animal feed industries. The gene underlying this trait was recently cloned and encodes an ethylene responsive transcription factor (Taketa et al. 2008).

21.2.1 Human Food

Currently barley is largely consumed by poor farmers in Northern Africa and Asia. The highest levels of barley consumption are in Morocco where farmers consume on average 54 Kg per annum. In the highlands of Ethiopia barley

accounts for over 60% of the food consumed. It is also grown extensively for human food in the Himalayas, particularly Nepal, in the mountainous regions of Yemen and in the Andes, notably in Columbia, Peru, Bolivia and Ecuador (Grando 2002).

Recently, barley has attracted increased attention in the developed world due to its relatively high content of non-starch carbohydrates. Different classes of non-starch carbohydrates have been found to stimulate the immune system, to reduce cholesterol levels and to be anti-tumourogenic (Mantovani et al. 2008). In barley, the levels of (1,3;1,4)- β -D-glucan in cell walls of the starchy endosperm can be 70% or more by weight. While barley, oat, and rye grains are all rich sources of (1,3;1,4)- β -D-glucan, wheat, rice, and maize have much lower concentrations. The genes encoding the enzymes involved in (1,3;1,4)- β -D-glucan synthesis were recently cloned from barley (Burton et al. 2006). A combination of genetic information and candidate gene analysis was used to isolate this group of genes. The chemical similarities between cellulose and (1,3;1,4)- β -D-glucans suggested that the (1,3;1,4)- β -D-glucan synthases might be members of one of the Cellulose synthase like (*Csl*) gene families (Fig. 21.1). In most plants the

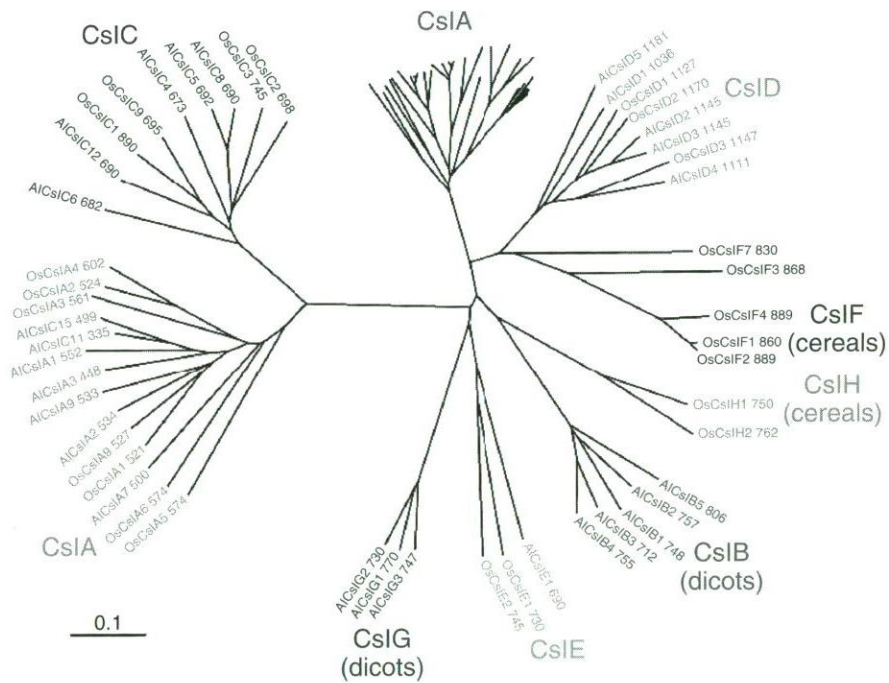


Fig. 21.1 The cellulose synthase (*CesA*) and cellulose synthase-like (*Csl*) gene families from higher plants contain around 50 members. The *CesA* genes are involved in cellulose biosynthesis but the functions of most of the *Csl* genes have not been defined (modified from Lerouxel et al. 2006) (See Color Insert)

Csl gene families are large and are divided into sub-groups, named *CslA* to *CslH*. Two groups, *CslF* and *CslH*, are only found in the cereals. Given that (1,3;1,4)- β -D-glucan is also only found in cereals, these two gene families appeared to be likely candidates for the synthases. By using comparative genomics and the known position of a major QTL affecting barley (1,3;1,4)- β -D-glucan levels, Burton et al. (2006) identified the *CslF* genes as the most likely candidates for (1,3;1,4)- β -D-glucan synthases. When the *CslF* genes were expressed in *Arabidopsis*, (1,3;1,4)- β -D-glucan was detected in walls of transgenic plants (Burton et al. 2006) confirming that the genes participated in the (1,3;1,4)- β -D-glucan synthesis.

The cloning of these genes offers the potential to modify the levels of (1,3;1,4)- β -D-glucan in barley and other cereal grains. High levels of (1,3;1,4)- β -D-glucan in wheat would be beneficial to human health, where they represent soluble dietary fibre and appear to reduce the risks of colorectal cancer, high serum cholesterol and cardiovascular disease, obesity and non-insulin dependent diabetes (Mantovani et al. 2008). Conversely, (1,3;1,4)- β -D-glucan is an anti-nutritional factor for monogastric animals and reduced levels would be desirable. Reduced levels are also desired by the brewing industry where (1,3;1,4)- β -D-glucan can cause problems in beer filtration. There may also be opportunities to use lowered levels of (1,3;1,4)- β -D-glucan in the biofuels industry. Therefore, the cloning of the genes involved in (1,3;1,4)- β -D-glucan synthesis provides the basis for the production of novel grains via genetic engineering.

21.2.2 Malting and Brewing

In barley, malting quality has been well studied and improved profiles have included whole grain qualities (colour and plumpness), rate of germination, specific enzyme levels to match the malting process, and thermo-stability of key enzymes.

A large number of components of malt quality are routinely screened in the quality assessment of malting barley. The most important are listed in Table 21.1 (see also Hamilton and Lewis 1974).

Malting and brewing essentially involves the enzymatic degradation of the barley endosperm and the fermentation of the released sugars. The first stage is the hydrolysis of the crushed cell layer surrounding the endosperm and of the cell walls within the endosperm. With the high content of (1,3;1,4)- β -D-glucan in the endosperm cell walls (1,3;1,4)- β -D-glucanase is of prime importance. There are two enzymes EI and EII that are responsible for this process. Proteases also play an active role in facilitating hydrolysis of the starch granules but are also involved in activation of other malt enzymes including protein Z, α -amylase and β -amylase. A recent transcript analysis that compared malt and feed barley identified carboxypeptidase as a key determinant of malt quality (Potokina et al. 2006).

Table 21.1 A summary of the major characteristics used to assess malt quality (from Eglinton 2003)

Malt quality parameters	Definition
Moisture (%)	Percentage of water in the malt
Extract, Fine (%)	Level of water soluble material extracted from the malt after different levels of milling
Extract, Coarse (%)	
Fine/Coarse Extract Difference (%)	Provides a simple index of modification
Wort colour	The intensity of the colour of the wort, reflecting the internal colour of the grain
Diastatic Power (DP)	Total activity of the enzymes involved in starch hydrolysis
Alpha Amylase	α -amylase enzyme activity extracted from malt
Beta Amylase	β -amylase enzyme activity extracted from malt
Fermentability (AAL%)	The level of fermentable sugars obtained from the malt
Total Nitrogen	Total nitrogen content of the malt, this is also a measure of total protein
Soluble Nitrogen	Total nitrogen content of the malt, this is also a measure of the extent of modification
Kolbach Index	Ratio of soluble N to total N, providing a measure of the extent of modification
Wort Beta Glucan	Total (1-3),(1-4) beta glucan in the wort
Wort Viscosity	Reflects the level of beta glucan and other soluble high molecular weight material

Proteinase inhibitors also play a role in controlling the rate of endosperm digestion. In particular, protein Z (a serine proteinase) has been implicated in influencing endosperm hydrolysis (Hejgaard et al. 1985).

21.2.2.1 β -amylase

Several enzymes are involved in the hydrolysis of the starch. These include α -amylase, limit dextrinase, α -glucosidase and β -amylase. The genes encoding these enzymes have all been cloned and several groups have investigated natural variation in the properties of these enzymes and the corresponding genes. However, in QTL studies β -amylase is the only enzyme that consistently shows an association with diastatic power, a key determinant of malt quality (see below). There is also a strong association between β -amylase activity and wort attenuation (the amount of un-degraded starch that remains due to incomplete hydrolysis).

There are two forms of β -amylase in barley. One is endosperm specific, accumulates during seed development and is encoded by *Bmy1* on the long arm of chromosome 4H. This enzyme is a major component of grain protein. The second enzyme is not as tissue specific, is encoded by *Bmy2* locus on chromosome 2H, and does not play a significant role in the malting processes. In most commercial malts β -amylase activity appears to be limiting although the efficiency of this enzyme is influenced by other enzymes involved in starch

degradation, particularly limit dextrinase (MacGregor et al. 1999). This issue is particularly important where barley malt is used in conjunction with adjuncts, usually derived from rice or maize. The quantity of β -amylase *per se* does not seem to be the major concern since this protein makes up 1–2% of the total barley protein in malt (MacGregor et al. 1971). It is more probable that variation in the thermostability and substrate affinity of β -amylase are responsible for the variation seen in diastatic power. During brewing, the malt passes through a mashing phase where the temperature can reach 70°C. β -amylase has maximal activity at 45°C but its activity starts to decline as the temperature goes above 55°C (Yoshigi et al. 1995). Three β -amylase alleles, *Bmy1*-Sd1, *Bmy1*-Sd2L and *Bmy1*-Sd2H are found in commercial barley varieties although several further alleles have been found in wild barleys. These have medium, low and high thermostability, respectively (Eglinton et al. 1998).

The various β -amylase alleles have been cloned and sequenced and active β -amylase enzyme can be readily produced in bacterial (*E. coli*) expression systems. Consequently, it has been possible to use site-directed mutagenesis to characterize the effects of each amino acid variant on the activity and thermostability of the enzyme (Ma et al. 2001). These results indicated that the variation in thermostability was due to two amino acid substitutions which changed the thermostability by around 2°C each (Ma et al. 2001). The variation in activity was also due to single amino acid differences. Based on these findings, Ma and colleagues (2001) generated a novel β -amylase gene that combined optimal specific activity and thermostability. The detailed structural information now available on the β -amylase enzyme and the ability to engineer new variants of this enzyme with enhanced properties for the brewing industry offers new options for genetic engineering of barley (Ma et al. 2000). While these options have been available for several years, they are yet to be translated to commercial reality. However, they have allowed researchers to develop sophisticated strategies for screening wild germplasm for new “natural” variants (Eglinton 2003).

21.2.3 QTL associated with malting quality

Hayes et al. (2001) reported that there are 181 QTL described in the literature for 29 barley and malt quality phenotypes. These are summarized on the web site <http://www.css.orst.edu/barley/nabgmp/qtlsum.htm>. This summary shows a BinMap created by Kleinhofs and Han (2002). Each chromosome has been divided into a number of bins and the QTL have been assigned to these bins. QTL for malt extract have been identified in 8 populations developed by researchers from all over the world. These are schematically shown in Fig. 21.2, which is based on this BinMap.

The most extensively studied population is Steptoe/Morex. Malt extract was first measured in this population in 1991 from four sites and this was repeated in 1992 at a further five sites (Hayes et al. 1993; Hayes and Iyamabo 1994;

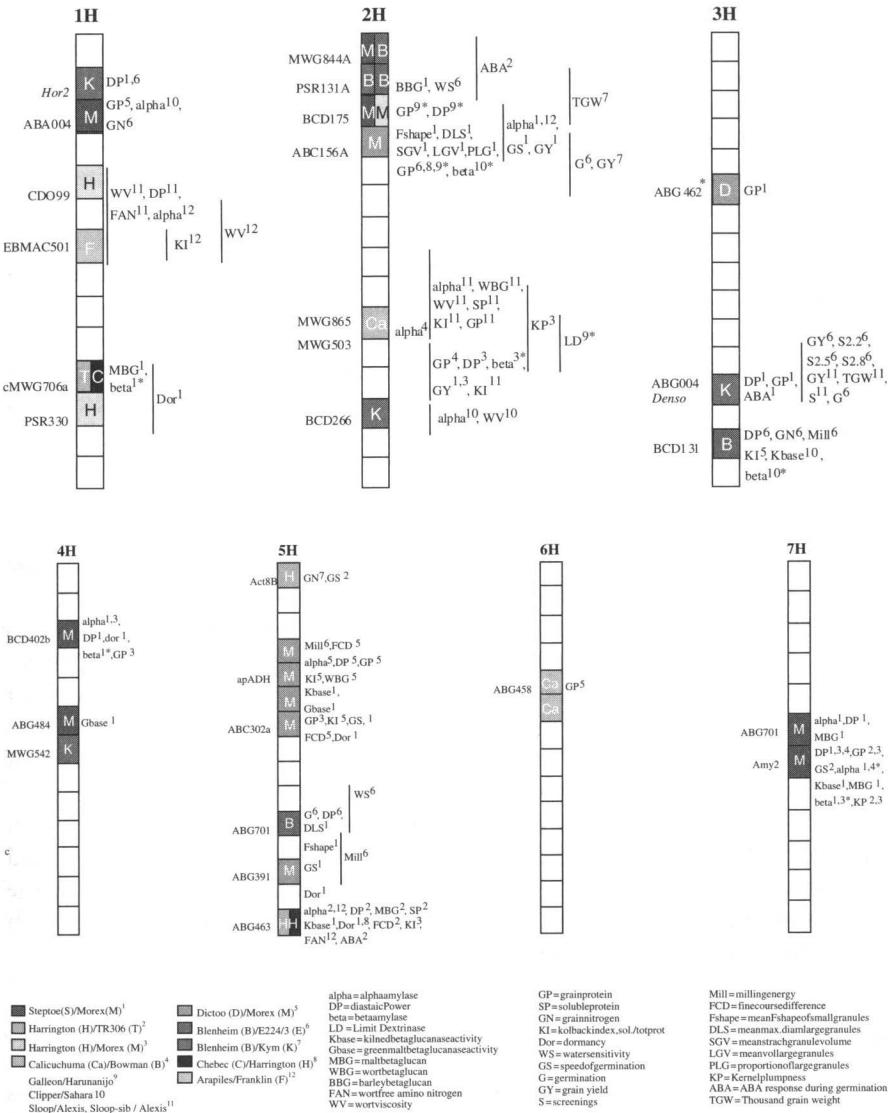


Fig. 21.2 A schematic representation of malt extract QTL, based on Hayes et al. (1997, 2001), <http://www.css.orst.edu/barley/nabgmp/qtlsum.htm>. Each chromosome is separated into a number of regions called Bins as described by Kleinhofs and Han (2002). Colored squares represent regions found in mapping populations around the world and indicate the locations of the malt extract QTL locations – the letters in the colored boxes indicate the first letter of the parent contributing the malt extract QTL allele. Markers are listed left of each chromosome. Listed right of the chromosome are other traits found to be associated with each region. As discussed in the text the populations analyzed to produce the distribution of QTL include (1) *Steptoe/Morex*: Two “six row” varieties, grown and mapped in USA. Steptoe is a low extract feed quality variety and Morex is a malting quality variety. (2) *Dictoo/Morex*: Two “six row” varieties. Also grown and mapped in the USA. Dictoo is a winter variety and Morex

Ullrich et al. 1997). QTL for malt extract were identified on all chromosomes except 3H. However, a number of these were only identified using data from individual sites and consequently have not been included in the summary by Hayes et al. (2001). All of the QTL in this summary, from this population, have Morex donating the higher malt extract allele (Fig. 21.1). However, Steptoe is responsible for two of the QTL that were identified using data from a single site only (Hayes and Iyamabo 1994).

Morex was also used as a parent to develop two other mapping populations, namely Harrington/Morex and Dictoo/Morex. Only one region, on the short arm of chromosome 2H, was found to be significantly associated with malt extract in all three of these populations. Morex is responsible for donating the higher malt extract allele in this region, for all three populations. Four other significant regions were also found to be associated with malt extract in the Dictoo/Morex population, three regions on chromosome 5H and one on chromosome 3H (Oziel et al. 1996).

Two other significant regions were found to be associated with malt extract in the Harrington/Morex mapping population, both on chromosome 1H (Table 21.1) (Marquez-Cedillo et al. 2000). Harrington is responsible for donating the high extract allele at these two regions. The region on the long arm of chromosome 1H is flanked by a region found to be associated with malt extract in two other populations with Harrington as a parent (Harrington/TR306 and Chebec/Harrington). However in both of these cases Harrington donated the low extract allele (Mather et al. 1997; Hayes et al. 2001; Collins et al. 2003).

A region was found to be associated with malt extract on the long arm of chromosome 5H in the two populations Chebec/Harrington and Harrington/R306 (Mather et al. 1997; Hayes et al. 2001; Collins et al. 2003). Harrington donated the high extract allele in both populations. Malt extract was also found to be associated with a region on the short arm of chromosome 5H, in the population Harrington/TR306. This region was not found to be associated with malt extract in any other population.



Fig. 21.2 (continued) is a spring variety. (3) *Harrington/TR306*: Two "two row" varieties. This population was grown and mapped in Canada. Harrington is a high extract malting variety and TR306 is a feed quality line. (4) *Harrington/Morex*: A cross between two high extract malting quality varieties. These two varieties are the "two row" and "six row" malting quality standards for North America. (5) *Calicuchuma-sib/Bowman*: Calicuchuma-sib is an ICARDA/CIMMYT "six row" variety; Bowman is a "two row" variety. This population was grown and mapped in the USA. (6) *Blenheim/E224/3* and *Blenheim/Kym*: These populations were grown in the UK. They are the only populations where the extracts were measured using the IOB method. (7) *Chebec/Harrington*: This was grown and mapped in Australia by the National Barley Molecular Marker Program (NBMMP; Barr et al. 2003; Pallotta et al. 2003). (8) Two other populations, developed in Australia, have been used for mapping malting quality traits but not malt extract. These are Galleon/Haruna Nijo and Clipper/Sahara (Karakousis et al. 2003a,b) (*See Color Insert*)

The other two populations with a common parent are Blenheim/E224/3 and Blenheim/Kym (Thomas et al. 1996; Bezant et al. 1997; Powell et al. 1997). Thomas et al. (1996) found 18 regions in the Blenheim/E224/3 mapping population that were associated with malt extract, and another 22 regions that were associated with malt extract when adjusted to a grain nitrogen level of 1.5%. Of these, only four regions were found to be associated with malt extract at more than one site. Powell et al. (1997) found a further three regions associated with malt extract in that population, one of which was in common with the regions Thomas et al. (1996) found. Three of these regions could not be assigned to a bin and are not shown in Fig. 21.2. Although the variety Kym generally has a lower malt extract than Blenheim, it was responsible for donating the higher-level allele at five of the eight regions found to be associated with malt extract in the Blenheim/Kym population (Bezant et al. 1997). Three regions could not be assigned to a bin and are not shown in Fig. 21.2. Only a single region was found to be significantly associated with HWE in the Blenheim/E224/3 and Blenheim/Kym populations (Thomas et al. 1996; Bezant et al. 1997; Powell et al. 1997). This region is on the short arm of chromosome 2H and has Blenheim donating the higher malt extract allele.

QTL have been found for many other traits associated with malting quality (see Fig. 21.2). A single region of the genome is often found to influence a number of different traits. The terminal chromosome 5HL region, for example, carries major genetic factors for seed dormancy, pre-harvest sprouting, high malt extract, diastatic power, alpha amylase, wort free amino nitrogen, soluble protein and reduced levels of malt β -glucan and fine course difference. Dissecting the details of this region at the genomic level is of particular interest. Whether this region contains a gene "cluster", number of closely linked individual genes controlling each trait separately or a single gene that has pleiotropic effects on each trait is currently unknown. A particular gene of interest located in this region is GA 20-oxidase, as discussed in Section 21.2.4.

In total more than 24 individual chromosome regions have been found to be associated with malt extract (Fig. 21.2) and the use of molecular markers linked to these regions can improve the effectiveness of selection of new varieties in barley breeding programs.

21.2.4 Germination as a Key Variable in Barley Quality

Susceptibility to preharvest sprouting of the grain while it is still in the head has become a significant challenge since the dormancy required to combat this susceptibility is detrimental to the germination required for the malting process. The challenge for breeders is to develop a barley genotype that is dormant enough to withstand preharvest sprouting but not too deeply dormant so that the grain does not germinate during the malting. Generally, there are two main phenotypes in the expression of dormancy, those that release dormancy

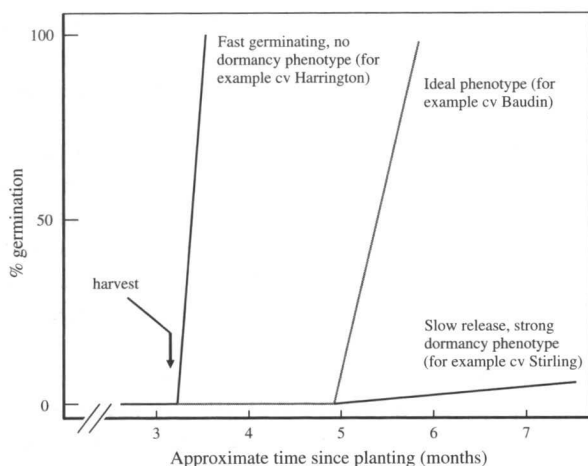


Fig. 21.3 Hypothetical graph demonstrating the germination behaviour of a fast germination phenotype with no dormancy such as the Harrington cultivar, a slow release phenotype with strong levels of seed dormancy such as Stirling and what may be the ideal seed dormancy phenotype displaying strong dormancy with a sudden timely release of dormancy such as Baudin (See Color Insert)

gradually and those that remain dormant for several months with a sudden release in dormancy. Ideally, a barley malting cultivar would be deeply dormant (primary dormancy) for the months during grain filling with a sudden release of dormancy immediately post harvest to reduce the cost of storage time (Fig. 21.3).

The QTL analyses performed on the Stirling/Harrington population in Bonnardeaux et al. (2008) identified two seed dormancy QTL on the 5H chromosome, that were consistent with the SD1 and SD2 QTL detected in several seed dormancy studies using feed varieties (Gao et al. 2003; Han et al. 1996, 1999; Oberthur et al. 1995). The SD1 and SD2 loci have been proposed to have different physiological functions, with SD1 affecting the formation and maintenance of dormancy and SD2 associated with the release of dormancy (Prada et al. 2004). The near-centromeric QTL on 5H (SD1) has been reported as the major QTL in previous studies and the QTL on the long arm of 5H (SD2) as a minor QTL. In some studies, however, the major QTL is on the long arm (SD2, Bonnardeaux et al. 2008). Therefore, the SD2 QTL may be more relevant to malting varieties and Australian germplasm. In a recent study Lin et al. (manuscript in press), mapped the SD1 and SD2 QTL for seed dormancy from a cross of Morex/Harrington and in contrast to the non-dormancy allele normally being derived from Morex (Han et al. 1996) the Morex allele was the dormant allele in this population. Thus, the nature of the QTL for seed dormancy on chromosome 5H needs further study. Li et al. (2004) conducted a comparative genomics study using rice, wheat and barley in the region of the SD2 locus on the long arm of 5H and suggested the gibberellin (GA) 20-oxidase

(encoded by the GA5 gene in Arabidopsis) was the underlying gene for this QTL. Since then, further evidence implicating the role of GA 20-oxidase in seed dormancy has emerged. Appleford et al. (2006) observed high levels of expression of the enzyme as well as GA 3-oxidase, a member of the same gene family, in developing and germinating wheat grains. In addition the GA 20-oxidase homologues are located in map positions in wheat chromosomes 5BL, 5DL and 4AL, syntenic to the region of the 5HL QTL in barley and consistent with an involvement in seed dormancy (Li et al. 2004; Appleford et al. 2006). The GA 20-oxidase is therefore a clear candidate gene for the control of dormancy. Another candidate gene is the *Viviparous1* gene (*Vp1*, Hobo et al. 1999), equivalent to the *ABI3* transcription factor in Arabidopsis, and is located within the dormancy QTL region on chromosome 3 of wheat (Bailey et al. 1999; McKibbin et al. 2002) which is also found on 3H of barley. The *Vp1* product is involved in the regulation of ABA signaling (Hattori et al. 1992; Susuki et al. 2003; reviewed in Finkelstein et al. 2008).

Environmental factors, such as moisture in particular, could be expected to have their greatest influence following the release of primary dormancy and thus affecting after-ripening or when the seed is cycling between secondary dormancy and a quiescent state. Germination of seeds in these phases predominantly relies on favorable environmental conditions. Minor QTL or those detected only in certain environments may represent genes that are more responsive to the environment and become active in specific environmental conditions. Lee et al. (2002) characterized the first gene connecting environmental and endogenous interactions in seed germination. The Arabidopsis RGL2 gene encoding a transcription factor, is induced by moisture and commences signaling by GA (Lee et al. 2002). Identification of genes such as the RGL2, that act at the interface between environmental cues and hormone response would elucidate the pathways by which environmental factors influence variation in germination (reviewed in Finkelstein et al. 2008).

21.3 Genomics of Wheat Quality

Biochemical and genetic studies have indicated that the properties of a group of well defined wheat storage proteins largely determine the dough rheological properties (Wall 1979; Branlard and Dardevet 1985; reviewed in Gras et al. 2001; Howitt et al. 2003) of wheat flour, a key quality attribute. Modification of quality can occur in a predictable way based on the genetic complement or in an unpredictable way as a result of environmental conditions during kernel maturation. In most dicotyledonous, and some monocotyledon seeds, the globulin types predominate in the grain. However, in the Triticeae (wheat, barley, rye, triticale) the major portion of seed proteins are not globulins but classes of protein characterized by regular repetitive domains with unique and fundamental functional features that determine wheat quality (Macritchie et al. 1990; Shewry and Halford 2002) and variation in these glutenin proteins either

quantitatively or qualitatively has major effects on end-product quality. The predictive power of defining the complement of glutenin subunit proteins for flour processing properties in breeding populations has been demonstrated for difficult traits such as extensibility (Eagles et al. 2002, 2004).

The physical properties of dough play a large role in determining its functionality. The extent to which a piece of dough can be stretched, and the force required to do so helps to determine the suitability of a variety for specific end-uses (Simmonds 1989; Eliasson and Larsson 1993). For example, leavened bread is best produced from dough that possesses strong and balanced rheological properties. In contrast, dough for biscuit production is generally less resistant to extension but is able to be extended a large distance before rupturing. This allows the dough to flatten and spread into a large, flat, evenly shaped biscuit (Simmonds 1989). Dough rheology is often measured using either an Extensograph (Brabender, Germany) or an Alveograph (Chopin). In both cases, slow sample throughput, the requirement for large sample sizes and the impact of extraneous error hamper genetic gain for improved rheological properties in wheat.

Strong dough will form a cohesive mass that has resistance to extension and is stable during mixing (Simmonds 1989). Such dough is able to hold the gas produced during fermentation within evenly distributed discrete cells and generates a loaf crumb with a much valued appearance and texture (Simmonds 1989). Such a crumb structure appears light in color, fine and silky in structure, both highly desirable quality attributes. Soft gluten will allow the gas cells to expand excessively during fermentation, causing their walls to collapse and the cells to coalesce together. The resulting bread has an undesirable, coarse, structure resulting from a very uneven texture (Simmonds 1989; Finney et al. 1987).

21.3.1 The Wheat Flour Proteins

Traditionally four different groups of proteins have been identified in wheat flour: (1) albumins (soluble in water and dilute buffers); (2) globulins (not soluble in water but soluble in saline solutions); (3) prolamins (which are soluble in 70–90% ethanol); (4) glutenins (which are soluble in dilute acid or alkali) (Beccari 1745; Osborne 1907). The glutenins derive from the gluten polymer and are usually subdivided into two distinct groups reflecting their solubility in 70% ethanol, namely glutenin and gliadins (Wrigley et al. 1996; Shewry and Casey 1999). Gliadins are usually single polypeptide chains (monomeric proteins) and the glutenins are multichained structures of polypeptides that are held together by disulfide bonds. The very high molecular weight of the polymeric structures formed by glutenins is responsible for their distinct contribution to the dough processing in contrast to gliadins which do not form large polymers (Fig. 21.4). Therefore, the classification of these proteins into monomeric and polymeric forms is a good indicator of dough functional properties (Payne and Lawrence 1983).

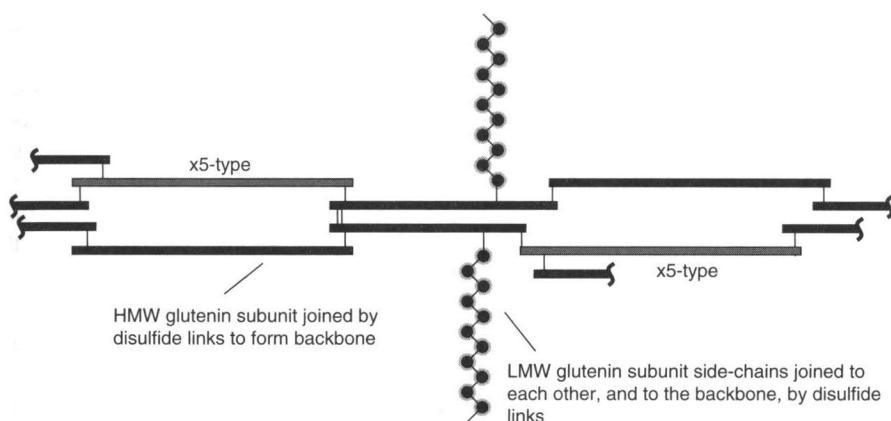


Fig. 21.4 Diagrammatic representation of the gluten polymer. The high molecular weight (HMW) glutenin subunit shown in red is particularly active in forming disulfide based polymer structures because it has an additional cysteine residue available for inter molecular linkages. The vertical entities in the figures are the low molecular weight (LMW) glutenin subunits. The diagram is based on Miles et al. (1991) and Wieser (2007) (See Color Insert)

The investigation of glutenin proteins in relation to dough properties have indicated two key variables, (1) the nature of the protein allele (reviewed in Gras et al. 2001) and (2) the level at which the respective allele is expressed (Butow et al. 2003). In some cases the control of the level of expression of seed storage protein has been well studied (reviewed in Appels et al. 2003; Ravel et al. 2006) but in general the interaction between promoters controlling gene expression and the environmental conditions during grain maturation have not been analyzed in any detail. The DNA structures of most of the glutenin genes have been determined and in some cases the genomic regions carrying the genes have been sequenced (Anderson et al. 2003; see also Section 21.3.2).

The prolamins forming the polymer are mainly the high- and low-molecular-weight glutenins, while the monomeric (polymer non-participating) prolamins are called α -, γ -, and ω -gliadins. In addition to the prolamins, other major wheat seed proteins include a wide assortment of proteins variously classified as globulins/albumins – including the starch granule associated grain-softness-protein and puroindolines, CM (chloroform-methanol soluble) proteins, amylase- and proteinase-inhibitors, thionins, and numerous other proteins of unknown function. Many of these additional seed proteins retain conserved motifs, such as cysteine numbers and placements, suggesting a common evolutionary source with the prolamins – including a number of proteins similar to gliadins but lacking the prominent repetitive domains characteristic of the wheat prolamins and tentatively named LMW-gliadins (Anderson et al. 2001; also referred to as foam stabilizing proteins, Clarke et al. 2001, 2003). All of these proteins have a common ancestry along with many dicot seed proteins and can be considered members of a large and diverse superfamily. Why the prolamins arose to prominence in the Triticeae is not

known but could be either an, as yet, unknown selective advantage or simply a random result of seed protein divergence.

Within the wheat seed prolamins, two evolutionary lines have resulted in the major prolamin classes, with both lines originating separately within the superfamily of related seed proteins. From one line the LMW-glutenins and gliadins emerged – sharing enough similarities to establish a common origin. The major distinguishing feature of the LMW-glutenins from the gliadins is the presence of two cysteine residues available for intermolecular disulfide bonds – the basis of incorporation into the gluten polymer. The gliadins are considered monomeric due to the general absence of free cysteines. In the cases of the α - and γ -gliadins, the even number of cysteines form intramolecular disulfide bonds, while the ω -gliadins generally lack cysteine residues altogether. Although not major participants in the gluten polymer, the monomeric gliadins contribute to dough physical/chemical properties by extensive hydrogen bonding with the gluten polymer (Macritchie et al. 1990; Shewry and Halford 2002).

Several gliadin genes have been reported to contain an uneven number of total cysteines, thus potentially leaving one available for intermolecular cross-linkages (Lindsay and Skerrett 2000); γ -gliadin (Sugiyama et al. 1986; Ferrante et al. 2006), and ω -gliadin (Altenbach and Kothari 2007) are examples. The effect of these gliadins on wheat quality parameters is unclear, but gliadins and LMW-glutenins with an uneven number of cysteines would be “chain-terminators” since they would lack a second free cysteine to continue the polymerization process (D’Ovidio and Masci 2004).

The second evolutionary line of wheat prolamins contains only the HMW-glutenins, and likely arose as a tandem duplication of a globulin gene, with one of the resulting genes evolving into a HMW-glutenin (Kong et al. 2003). A subsequent tandem duplication of the chromosome fragment containing an ancestral HMW-glutenin and globulin genes resulted in the x- and y-type HMW-glutenin genes.

The two HMW-glutenin genes have remained as two conserved genes, while the gliadins and LMW-glutenin genes have radiated into large multi-gene families of highly variable member copy numbers. Estimates of gliadin/LMW-glutenin gene family sizes have varied from 50 to 150 (Anderson et al. 1997) to 300 (Okita et al. 1985) for the α -gliadins, and 30–40 for the LMW-glutenins (Sabelli and Shewry 1991; Cassidy et al. 1998). Fewer estimates have been attempted for the γ - and ω -gliadin gene families, but estimates that are consistent with protein studies indicate 20–40 γ -gliadin genes and 8–15 ω -gliadin genes. Such estimates are confounded by a high percent of inactive pseudogenes (Anderson 1991; Anderson and Greene 1997; D’Ovidio and Masci 2004) – a characteristic of cereal prolamins.

The complication in estimating active gene numbers is particularly striking in the region of the B-genome ω -gliadins (Gao et al. 2007) where large numbers of gene fragments are found. In this region there are eight ω -gliadins sequences, only two of which appear to code for expressed proteins, with the other six either full-sized pseudo-genes or truncated fragments of ω -gliadin sequences.

In examining available LMW-glutenin sequences, Ikeda et al. (2002) proposed 12 distinct LMW-glutenin gene types. A careful examination of ESTs suggests restricting that further to perhaps only 9 active LMW-glutenin genes in hexaploid cultivars (O. Anderson, unpublished). In such estimates, identical gene sequences resulting from recent gene duplications are not accounted for. Together, such findings suggest reducing estimates of the active wheat gliadin/LMW-glutenin gene family sizes to one-quarter to one-half of estimates based solely on Southern hybridization experiments, to 20–70 for the α -gliadins, 8–15 for the γ -gliadins, 4–6 for the ω -gliadins, and 8–15 for the LMW-glutenins.

21.3.1.1 High Molecular Weight Glutenin Subunits (HMWGS)

The HMW glutenins are relatively minor components in terms of quantity, but they are major determinants of gluten elasticity through promoting the formation of larger glutenin polymers and thus are key factors for bread-making (Tatham et al. 1985). They are encoded by the *Glu-1* loci *Glu-A1*, *Glu-B1*, and *Glu-D1* that are located on the long arms of chromosome 1A, 1B and 1D, respectively. Each locus includes two genes linked together encoding two different types of HMW glutenin subunits, *x*- and *y*-type subunits (Payne et al. 1981, 1987; Shewry et al. 1992). The *x*-type subunits generally have a higher molecular weight than the *y*-type subunits. Payne and Lawrence (1983) summarized the number of alleles at the *Glu-1* loci: three allelic forms for *Glu-1A*, eleven alleles for *Glu-1B*, and six alleles for *Glu-1D*. Later, more alleles have been identified as reported by McIntosh et al. (2003); see also Liu et al. (2003); Sun et al. (2006). Although six genes exist for HMW glutenin subunits, most common wheat cultivars possess only three to five HMW glutenin subunits (one to three subunits in durum wheats) due to gene silencing. All hexaploid wheats contain at least the *Bx*, *Dx*, and *Dy* protein subunits in their endosperm, while most cultivars also contain a *By* subunit and a *Ax* subunit as well. The gene encoding the *Ay* subunit is usually silent.

The *x* and *y* subunits share a highly similar primary structure, which consists of a signal peptide (removed from the mature protein), a N-terminal domain, a central repetitive domain, and a C-terminal domain (Shewry and Halford 2002). Most of the reported *x*-type subunits possess four conserved cysteine residues (three in the N-terminal domain, one in the C-terminal domain), and the majority of the *y*-type subunits characterized thus far contain seven conserved cysteine residues (five in the N-terminal domain, one in the repetitive domain and one in the C-terminal domain, Shewry and Tatham 1997). These cysteine residues are involved in the formation of disulphide bonds within and between subunits and are thus important for the high order structure and the functionality of these proteins in shaping the elastic properties of the gluten complex in wheat dough (Shewry and Tatham 1997; see also Fig. 21.4). In both *x*- and *y*-type subunits, the repetitive domains are composed of short and repeated peptide (tripeptide, hexapeptide, nanopeptide) motifs, with the presence of the tripeptide motif being unique to the repetitive domain of *x*-type

subunits (Shewry and Tatham 1997). With unusually high content of glutamic acid, HMW glutenin subunits also have high contents of proline and glycine and low contents of lysine. The amino acid composition of HMW glutenin subunits reveals the hydrophilic nature of the central repetitive domain and the hydrophobic characteristics of the N- and C-terminal domains (Shewry et al. 1989). The proportion of the different amino acids is mainly defined by sequences of repeated polypeptide motifs. Variations on the consensus repeat sequences PGQGQQ and GYYPTSPQQ form >90% of the repetitive domain (Anderson and Green 1989; Shewry and Tatham 1997).

Variations of the number of cysteine residues of HMW glutenin subunits have been associated with different properties in bread-making. For example, the *Dx5* subunit has an extra cysteine residue located at the N-terminal part of its repetitive domain and this subunit has frequently been found to associate with improved processing quality in bread wheat varieties (Lafiandra et al. 1993; Gupta and MacRitchie 1994; see also Fig. 21.4). A novel variant of the *Ax2** subunit, *Ax2*B*, is found to contain an extra cysteine residue located in the middle of its repetitive domain (Juhász et al. 2003). This subunit exerts a positive effect on the gluten properties. In contrast, the *Bx14* and *Bx20* subunits have reduced numbers of cysteine residues in their N-terminal domains (Shewry et al. 2003).

Increased dough resistance can also derive from an increase in expression of the *Bx7* subunit (Butow et al. 2003; *Glu-B1* locus (*al* allele)) compared to alternative alleles at that locus (Eagles et al. 2004). Structurally, the gene conferring *Bx7* over-expression has an 18 bp insertion in the central repetitive domain relative to the normal *Bx7* gene, and this feature has been used to develop PCR markers for differentiating these two genotypes. Other allelic variant pairs had similar results: *Glu-B1* subunits 17 + 18 (strong) versus subunits 20x + 20y (weak), (Cornish et al. 2001).

21.3.1.2 Low Molecular Weight Glutenin Subunits (LMWGS)

Most of the LMW glutenin subunits are encoded by the complex *Glu-3* loci (*Glu-A3*, *Glu-B3* and *Glu-D3*) on the short arms of chromosomes 1A, 1B and 1D (Gupta and Shepherd 1990; Jackson et al. 1983), though other LMW-GS gene loci were also reported, such as *Glu-B2* and *Glu-B4* on chromosome 1B (Jackson et al. 1985; Liu and Shepherd 1995), *Glu-D4* on chromosome 1D and *Glu-D5* on chromosome 7D (Sreeramulu and Singh 1997). Six, eleven and five alleles defined by protein electrophoretic mobility have been confirmed at *Glu-A3*, *Glu-B3* and *Glu-D3* locus, respectively, in common wheat (Branlard et al. 2003; Gianibelli et al. 2001; Gupta and Shepherd 1990). Recently, some new alleles such as *Glu-B3m*, *Glu-B3n* (McIntosh et al. 2003) and *Glu-D3f* (International Maize and Wheat Improvement Center and Japan NARC, unpublished) have been designated.

Based on the first amino acid present in the N-terminal sequences of the proteins, seven main types of LMW glutenin subunits have been identified,

which are LMW-s starting with the sequence SHIPGL-, three LMW-m with N-terminal sequences of METSHIPGL-, METSRIPGL and METSCIPGL-respectively, and three types with N-terminal sequences resembling those of the α -, β - and γ -type gliadins (Kasarda et al. 1988; Tao and Kasarda 1989; Lew et al. 1992; Cloutier et al. 2001). LMW glutenin subunits were further classified into 12 groups by Ikeda et al. (2002) according to deduced amino acid sequences and in particular the number and position of cysteine residues available for inter-molecular disulphide bond formation (Shewry and Tatham 1997). More than 100 genes, partial genes and pseudogenes of the LMW-GS family have been cloned and sequenced from several common wheat cultivars (Pitts et al. 1988; Cloutier et al. 2001; Ikeda et al. 2002; Zhang et al. 2004). Hai et al. (2005) retrieved 69 known LMW-GS genes from GenBank and classified them into nine groups by the deduced amino acid sequence of the highly conserved N-terminal domain, and nine corresponding primer sets proved to be LMW-GS group-specific were established. Ikeda et al. (2006) also constructed 10 group-specific markers according to the published nucleotide sequences. Zhao et al. (2006, 2007) analysed a set of Australian wheat cultivars and identified 6 different gene sequences and 12 gene haplotypes at the *Glu-D3* locus.

21.3.2 Seed Storage Protein Gene Structure and Variation

In spite of the large amount of literature on the wheat prolamins, there is still an incomplete understanding of the chromosomal organization of these genes and loci, but what is known gives insight both to the prolamin genes and global characteristics of the wheat genome. The HMW-glutenins have the simplest organization since there are only two genes per genome, and this region is the best studied area of the wheat genome (Gu et al. 2006). The immediate gene complement around the HMW-glutenin *Glu-1* loci is shown in Fig. 21.5A. The two HMW-glutenin genes are separated by 51–185 kb in the genomes thus far examined, with the variation in distance due to completely different complements of nested transposons among the A, B, and D genomes (Gu et al. 2006). In only the D genome have the two HMW-glutenin genes at the *Glu-1D* loci from both *T. tauschii* and *T. aestivum*, been found on a single BAC (Anderson et al. 2003; Gao et al. 2007). For all cases of the *Glu-1A* and *Glu-1B* loci, the spacing was sufficient to require two or three BACs to confirm spanning the HMW-glutenin intergenic regions of the A and B genomes: tetraploid (Gu et al. 2004), hexaploid (Gu et al. 2006), and *T. monococcum* (Gu et al. unpublished). The pattern of genes is conserved in all genomes studied including diploid, tetraploid, and hexaploid wheats; i.e. the locus contains a receptor kinase, globulin, γ -type HMW-glutenin, remnants of a globulin, α -type HMW-glutenin, and a protein kinase (Fig. 21.5A).

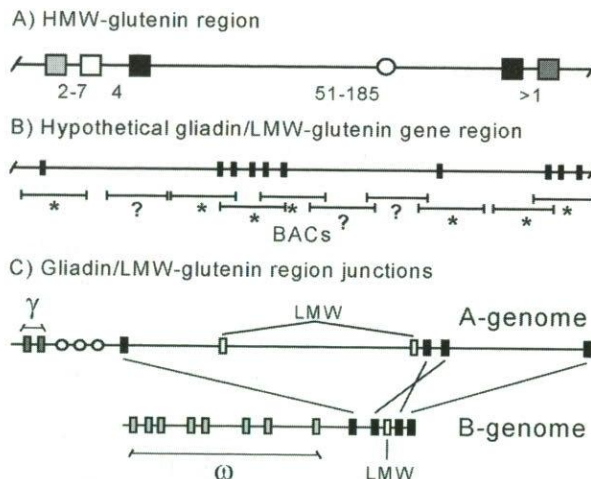


Fig. 21.5 Structure of wheat prolamins. The identity and relative spacing of examples of wheat prolamins is shown. **(A)** HMW-glutenin region. All wheat HMW-glutenin regions have the same gene order; i.e., a receptor kinase (light grey box), a globulin (white box), the y-type HMW-glutenin (black box), fragments of a globulin (white circle), the x-type HMW-glutenin (black box), and a protein kinase (dark grey box). Variations in intervening sequences are shown in kilobases below gene pairs or between the two HMW-glutenins. **(B)** Generic gliadin/LMW-glutenin loci structure. A general model of the gliadin/LMW-glutenin loci structure is diagrammed to be small clusters of genes separated by longer sequences without prolamins. Possible BAC locations are shown under the loci to indicate how BACs isolated via prolamins probes may not span the entire loci space. Asterisks mark BACs containing prolamins. Question marks indicate BACs not containing prolamins and which span gaps in BAC contigs. **(C)** Comparing two BAC sequences containing common markers but different prolamins classes and gene orders. Common markers are black boxes. LMW-glutenin genes are white boxes. Dark grey boxes indicated γ -gliadin genes, and light grey boxes indicate ω -gliadin sequences. White circles are three genes related to the major prolamins classes but not classified

The gliadin/LMW-glutenin gene families are generally composed of larger numbers of genes – the exception being the *Glu-3A* locus, which contains 2–3 genes with only one gene likely to be functional (Wicker et al. 2003; Gao et al. 2007; Anderson unpublished). The other loci are structured with multiple genes spread over longer distances and with varying clustering of genes. Several studies have attempted, with limited success, to isolate BAC contigs over extended wheat genomic regions using specific known probes; i.e., LMW-glutenin/gliadins (Ozdemir and Cloutier 2005; Gao et al. 2007), and the *Ph1* region (Griffiths et al. 2006). In another study, four randomly chosen wheat BACs had gene densities of about 1 gene/75 kb (Devos et al. 2005). A similar average spacing was found when sequencing megabase-sized regions on the 3B chromosome (Choulet et al. 2008), and unpublished findings are that as many as one-third of wheat BACs are without genes. The many examples of relative clustering of small numbers of wheat genes into gene “islands”, the failure to

identify long BAC contigs using gene probes, and the known incidences of BAC+ sized regions absent of genes, together suggests the organization of the larger prolamin gene family regions is proposed as shown in Fig. 21.5B – islands of singlets and clusters of prolamin genes separated by stretches of repetitive DNA. As shown in Fig. 21.5B, probing with prolamin sequences will identify those BACs carrying prolamin genes and allow singleton/contig identification (BACs marked with astericks in Fig. 21.5B) but would not identify intervening BACs without prolamin genes – resulting in a number of smaller contigs, but no single contig covering the entire locus. The larger of these loci could be very large. In the case of the α -gliadin gene family, previous results suggest the *Gli-3A* locus could contain as many as 100 genes (active genes plus pseudogenes). With an average gene spacing of 75 kb, this would make the *Gli-3A* locus 7–8 Mb in size in some cultivars.

Although there is limited data available on the DNA sequence structure adjoining prolamin loci, the available results suggest major differences among the wheat genomes. Similar to the study on the wheat α -gliadin loci by Gu et al. (2004), Gao et al. (2007) isolated LMW-glutenin, γ -glidin, and ω -gliadin containing BACs from tetraploid wheat. As with the α -gliadin study, contigs within each prolamin family showed small clustering of genes interspersed with longer DNA stretches generally precluding assembling complete contigs of these loci. However, two BACs, one each from A and B genomes, contained representatives of two different prolamin classes; i.e., LMW-glutenin and γ -glidin in one case, and LMW-glutenin and ω -glidin in the other. The initial assumption was that the junctions of these adjacent prolamin multigene families had been isolated with the two gliadin loci on opposite sides of the LMW-glutenin locus in the two genomes. Sequencing of the two BACs subsequently showed that the situation was in fact more complex than previously appreciated (Fig. 21.5C). Four markers are in common between the two sequences (black boxes and connecting lines in Fig. 21.5C), indicating common ancestry, but the order and identity of the other genes is different. In addition to the four common markers, the A-genome sequence contains the complete *Glu-A3* locus (two LMW-glutenin genes) plus two γ -glidin genes separated from the LMW-glutenin genes by three genes related to prolamins but not classifiable into the standard wheat prolamin classes. In the B-genome, the sequence includes a single LMW-glutenin bracketed by the common markers, with eight genes and gene fragments for ω -gliadins immediately adjacent – but no prolamin-related genes as in the A-genome. Figure 21.5C implies the contradiction that the two different gliadin loci are situated in the same orientation and adjacent with respect to the LMW-glutenin loci. The explanation may include features such as interspersion of the prolamin genes, local inversions, and other as yet unrecognized differences in organization.

21.3.2.1 Assaying Variation in Seed Storage Proteins

Glutenin alleles can be identified by extracting the glutenin proteins from seed and separating them on SDS PAGE gels (Singh et al. 1991). Although capable

of detecting a wide range of alleles at the six glutenin loci, this method is slow and expensive, limiting its use to advanced breeding material. MALDI-TOF based analysis of seed storage proteins is a technology that is capable of high resolution as well as high throughput (Zolla et al. 2002; Chen et al. 2007). Alternatively, DNA based molecular markers specific for particular glutenin alleles, have been developed to aid in selection for improved dough rheology (D'Ovidio et al. 1994; Devos et al. 1995; Ahmad 2000; Juhász et al. 2003; Radovanovic and Cloutier 2003; Zhang et al. 2004; Gale 2005). PCR-based assays to distinguish different HMW glutenins have been established. Sets of PCR primers can now amplify the complete coding region of the major HMW glutenin coding regions, including the *Ax*, *Bx*, *By*, *Dx* and *Dy* coding regions (D'Ovidio et al. 1994, 1995, 1996). PCR-based assays to specifically detect the *Dx5* gene (D'Ovidio and Anderson 1994) and to distinguish *Dy12* from *Dy10* (Smith et al. 1994) have been developed. Since *Dx5-Dy10* (*Glu-D1d*) and *Dx2-Dy12* (*Glu-D1a*) are the two predominant alleles found at the *Glu-D1* locus, the *Dx5* assay (D'Ovidio and Anderson 1994) has been adapted to permit rapid discrimination between these alleles (Varghese et al. 1996). De Bustos et al. (2000) used a PCR procedure based on minor nucleotide sequence variation of the regions immediately flanking the gene sequences of various HMW glutenin alleles to selectively amplify the entire coding regions of *Ax2@*, *Ax1* or *Ax* Null, *Dx5* and *Dy10*. PCR-based markers specific for the *Dx5* and *Dy12* genes and a co-dominant marker for distinguishing between *Bx7* and *Bx17* have been developed (Ahmad 2000). A multiplex PCR was performed with these primers but since the size of the codominant *Bx* marker was well above 2 kb, while markers for *Dx5* and *Dy12* were below 700 bp, the multiplexing was only successful with markers for *Dx5* and *Dy10*. A set of PCR markers targeting all three HMWGS loci were developed by Ma et al. (2003) and these markers were successfully amplified together to systematically identify major alleles at the three loci by one PCR reaction. To date, a range of PCR markers are available to discriminate individual HMW-GS genes, including *Dx5*, *Ax1*, *Bx17*, *Bx7*, *Bx7*(OE), *By8*, *By8**, *By18*, *20x+20y* (*e* allele), (Ma et al. 2003; Butow et al. 2003; Lei et al. 2006). Recently, efforts have been made to establish the relationship between different protein mobility alleles and their corresponding allelic variants at DNA level. Based on the allelic variation of one LMW-GS gene at *Glu-A3* locus, sets of PCR markers were developed by Zhang et al. (2004) to identify *Glu-A3* protein mobility alleles. Zhao et al. (2006, 2007) identified 12 gene haplotypes and developed 7 STS-markers for *gluD3* alleles.

21.3.3 Flour Color

Flour color is the result of the yellowness of the endosperm and the absence of finely divided bran specks; the latter reflects the ease with which the aleurone and outer layers can be removed from the endosperm.

21.3.3.1 The Yellowness of Flour and Its End Products

The level of yellow pigment is usually recorded as b^* using a Minolta meter, results largely from variation of xanthophyll (mainly lutein) levels in the grain (Mares and Mrva 2001). Yellow flour is undesirable for bread manufacture, while flour for noodle production can vary from a creamy to yellow color depending on the style of noodle (Simmonds 1989). Yellow alkaline noodles (YAN) require a bright, clear yellow color that develops by the addition of alkali, while white salted noodles (WSN) require a bright white color (Simmonds 1989). For white bread and many other bakery products, the flour needs to be white.

Major genes controlling xanthophyll content and therefore yellowness of flour are situated on chromosomes 7AL, 7BL, 7DL wheat (Parker et al. 1998; Ma 1999; Francki et al. 2004). Smaller less significant associations with flour yellowness have also been detected on chromosomes 3AS, 3BS (Parker et al. 1998; Mares and Mrva 2001; Francki et al. 2004) as well as a number of other individual chromosomes. One of the genes coding for phytoene synthase has been collocated to the QTL on the group 7 chromosomes (Pozniak et al. 2007; He et al. 2007b) and is thus a good candidate gene for the control of lutein levels in the endosperm since it is directly involved in the terminal pathway that generates lutein. The intron-exon structure for the phytoene synthase on chromosome 7A of wheat was studied in detail by He et al. (2007b) and polymorphisms in this gene accounted for 20–28% of variation in yellow pigment across three environments. A mutation in the phytoene synthase gene on 7E (long arm) of tall wheatgrass has been shown to be genetically linked to yellow pigment content (Zhang and Dubcovsky 2008), confirming the importance of the gene in this phenotype. Zhang and Dubcovsky (2008) have also provided evidence for an unidentified gene near the phytoene synthase gene on 7AL that contributes to the control of yellow pigment accumulation in durum wheat. A second phytoene synthase gene was located to chromosomes 5A and 5B by Pozniak et al. (2007) although no QTL for color has yet been found on these chromosomes. Thirteen other genes are involved at various points in the biosynthetic pathway to form lutein and the comparative genomics approach to studying these genes is ongoing (Francki et al. 2004).

21.3.3.2 The Finely Divided Bran Specks in Flour

The presence of finely divided bran specks in flour is an issue in wheat quality when these specks become visible to the naked eye as a result of polyphenol oxidase (PPO) present in the bran. Bread made from dark flour, caused by the presence of bran in the flour, will generally have a small loaf volume, a coarser texture and darker crumb colour than that made from white flour (Simmonds 1989). The breakdown of lipids through lipoxygenase activity can also affect colour and cause off-flavours (Hessler et al. 2002); the *Lpx-B1* locus on chromosome 4BS is a major source of lipoxygenase activity.

The PPO enzyme is widely distributed in plant species (Flurkey 1989). The PPO enzyme catalyzes hydroxylation of monophenols to o-diphenols and oxidation of o-diphenols to o-quinones that polymerize non-enzymatically into dark color matter (Okot-Kotber et al. 2002). High PPOs in grain and flour is responsible for the undesirable time-dependent darkening of bread wheat (*Triticum aestivum* L.) based end-products such as noodles (Kruger et al. 1994a,b; Baik et al. 1995; Crosbie et al. 1996; Anderson and Morris 2003). PPO activity varies among wheat genotypes and is also affected by environment (Baik et al. 1995; Park et al. 1997; Ge et al. 2003). Cultivars with low PPO activity are desirable for the consumers and food manufacturers.

PPO genes have been cloned and sequenced in several plant species (Bucheli et al. 1996; Thipyapong et al. 1997). Recently, efforts have been made to clone PPO genes from bread wheat to understand the molecular mechanism underlying darkening of wheat based end-products (Demeke and Morris 2002; Jukanti et al. 2004). The sequence information of PPO genes has been used by Demeke and Morris (2002) to design oligonucleotide primers from the conserved copper binding regions of other plant PPO genes and thus obtained putative DNA sequence for the wheat PPO gene (GenBank accession number AF507945). Anderson (2004) obtained a PPO gene sequence from wheat cDNA library (GenBank accession number AY515506). In addition, sequence information of some other PPO genes was obtained from full-length sequencing of EST clones (Jukanti et al. 2004, GenBank accession number AY596266, AY596267, AY596268, AY596269, and AY596270).

Udall (1997) identified a QTL for PPO activity in a recombinant inbred line population derived from a cross between NY18 and CC, and found a RFLP marker Xcdo373 on wheat chromosome 2A closely linked to the QTL accounting for over 40% of the variation of PPO activity. Jimenez and Dubcovsky (1999) reported that genes located in the wheat chromosome homoeologous group 2 played an important role in PPO activity. Demeke et al. (2001) used three inbred line populations to study wheat PPO genes distribution, chromosome location, and number of loci involved in wheat PPO, and found polygenic inheritance in two populations (M6/Opata85, NY18/CC) and monogenic inheritance in the third population (ND2603/Butte86). They identified a QTL significantly associated with wheat PPO activity on chromosome 2D in the M6/Opata85 mapping population. Raman et al. (2007) also found a major QTL for PPO activity on chromosome 2AL in a DH population derived from Chara/WW2449 and suggested that the SSR markers Xgwm294 and WMC170 may be used for marker-assisted selection. Zhang et al. (2005) detected a QTL on chromosome 2AL closely linked to Xgwm312 and Xgwm294, explaining 38% of the phenotypic variance of grain PPO activity.

PCR-based markers for the PPO genes on 2A and 2D have been published (Sun et al. 2005; Raman et al. 2007; Massa et al. 2007; He et al. 2007a); the marker for the 2A gene is particularly diagnostic and amplifies a 685-bp and a 876-bp fragment in the cultivars with high and low PPO activity, respectively. The difference of 191 bp is located in the intron region of the PPO genes.

21.3.4 Flour Paste Viscosity

The high paste viscosity of flour is critical in determining the quality of Japanese white salted (Oda et al. 1980; Konik and Miskelly 1992) and Chinese noodles (Miskelly and Moss 1985). In breeding programs, both the visco-analyser and flour swelling volume tests have been used to determine the viscosity and therefore quality of wheat for noodle manufacture (Crosbie 1991; Panozzo and McCormick 1993). Biochemically, the ratio of amylose to amylopectin in starch is one of the major determinants of this viscosity (Sasaki et al. 2000). Yamamori et al. (2000) showed the important positive correlation between the quantity of granule bound starch synthase (GBSS) in wheat flour and flour amylose content. The location of a homoeologous gene series (*Wx-A1*, *-B1* and *-D1*) on chromosomes 7A, 4A (ancient translocation from 7B) and 7D (Chao et al. 1989), and the subsequent development of molecular markers to aid in the selection of the null alleles at each of these loci (McLauchlan et al. 2001), has provided an important tool for the improvement of noodle quality. Relatively few reports (Udall et al. 1999; Igrejas et al. 2002) exist of genetic associations with flour viscosity, other than the association with the *Wx* gene series (see also Section 21.4).

21.4 Grain Hardness and Carbohydrates in Wheat and Barley

The importance of carbohydrates has become evident as some clear genetic variation in starch and non-starch carbohydrates is being identified. Due to the strong relationship between the starch granules and protein matrix, hard textured varieties suffer greater starch damage during milling than soft textured varieties. Soft textured varieties are better suited to biscuit, cookie and some noodle manufacture while hard grained varieties are used for bread and some noodles (Simmonds 1989). While grain hardness is correlated with grain protein content (Giroux et al. 2000; Martin et al. 2001), genetic control of texture independent of protein content also exists.

The importance of starch in determining human health outcomes from the consumption of cereal based foods has been increasingly recognized and, in addition, cereals have been increasingly used as sources of energy in biofuels and/or animal feed (Topping 2007; Rahman et al. 2007). This appreciation of the significance of the carbohydrates in cereals has led to investigating the complexities of starch and non-starch polysaccharide structure through identifying the genes involved in their synthesis (Martinant et al. 1998; Shewry and Morell 2001; Morell and Myers 2005). The manipulation of cereal carbohydrates is now widely seen as one of the key areas for future innovation in grain quality.

21.4.1 Starch Content

Reductions in starch content associated with starch compositional change can lead to potentially useful alterations in other grain components (e.g. in the *Sex6*

mutant of barley; Bird et al. 2004a,b; Morell et al. 2003; Topping et al. 2003) but in commercial applications this means there is a tradeoff between yield penalty and added value. While there is some natural genetic diversity in starch content known in small grain cereals, the major research focus has been on increasing the flux to starch by manipulating the properties of the first committed step in starch biosynthesis, namely, the step carried out by the enzyme ADP-glucose pyrophosphorylase (see Meyer et al. 2007; Sakulsingharoj et al. 2004). Traits of importance for high starch applications include feed wheat, wheats for bioethanol production, wheats for starch/gluten separation.

21.4.2 Starch Composition

The advent of improved techniques for the isolation of genes and the relationship of those genes to expressed proteins and biochemical functions, have led to the cloning and isolation of a core set of genes in cereal quality (Morell and Myers 2005). Genome sequencing brought access to genome sequences in *Arabidopsis* and rice that allowed the remaining genes and candidate genes considered to be important in starch biosynthesis to be identified. Two further techniques have been important in allowing the knowledge of gene sequences and their expressed proteins to be harnessed. Firstly, the development of gene silencing techniques, such as antisense and RNAi, allow the role of key genes in hexaploid wheat to be directly tested (Regina et al. 2006; Fu et al. 2007). Secondly, techniques exist to generate and discover diversity in gene sequences, allowing the development of waxy wheat (Nakamura et al. 1995), wheats with deficiency in starch synthase IIa (Yamamori et al. 2000) and wheats lacking functional starch branching enzyme I, BEI, genes from each wheat genome (Regina et al. 2004). Techniques such as TILLING (Slade et al. 2005) provide the potential for identifying and combining mutations in each of the starch biosynthetic genes in each genome of wheat, and hence a comprehensive resource for identifying and designing, starches ideally suited to particular applications (Morell and Myers 2005).

Traits of importance for triple null wheat germplasm include waxy wheats and high amylose wheats and their commercial potential is being explored. Although triple null genotypes can be extreme in starch properties such as very low gelling temperatures and have significant yield penalties, they can provide valuable directions for the utilization of single and double null mutations. The latter can have important economic advantages driven by subtle variations in starch properties, and the added advantage of minimal impact on starch content or yield. The well known example is that of Udon noodles where mutations in GBSS result in high value segregations (Zhao et al. 1996; see also Section 21.3.4). Recently Konik-Rose et al. (2007) have demonstrated that subtle but important effects, on starch properties are associated with mutations in starch synthase IIa, SSIIa.

21.4.3 Non-Starch Polysaccharides

Cell-wall polysaccharides have long been known to be important in cereal quality. The term “pentosans” has been coined to describe the mix of arabinoxylans in cereals and the use of enzymes modulating the level and polymerisation state of pentosans is an important aspect of the bread improver market. The levels of pentosans are also important in feed applications for monogastric animals, particularly poultry. In comparison to starch, less is known of the genetic determinants of cell wall polysaccharides, mainly because of the difficulties in using biochemical approaches to identify the key enzymes, and because many of the genes involved are members of the very complex “Cellulose-Like” (Csl) gene family (Burton et al. 2007; see also Fig. 21.1). The genes included in this group include those involved in synthesis of a range of polysaccharides in addition to (1,3;1,4)- β -D-glucan and arabinoxylan. Although key traits include control of carbohydrate digestibility (e.g. glycemic index, metabolisable energy), manipulation of pentosans is also expected to impact water absorption and baking quality (Shewry and Morell 2001; Howitt et al. 2003).

21.4.4 Grain Hardness

The major locus involved in the control of grain texture (Symes 1965), *Ha*, is localized to the short arm of chromosome 5D (Mattern et al. 1973; Sourdille et al. 1996). Subsequently, two closely linked genes encoding puroindoline proteins (Gautier et al. 1994) were identified in the same region and have been considered to most likely encode the variation in grain hardness associated with the *Ha* locus (Giroux and Morris 1997). Giroux and Morris (1998) suggested that a mutation in the puroindoline-b gene (*Pinb-D1*), leading to an amino acid change, results in altered protein structure and consequently the strength with which the puroindoline protein binds with membrane polar lipids. This in turn was considered to alter the strength of the bond between the starch granules and protein matrix. In Giroux and Morris (1998) a null allele was also identified at the other puroindoline gene, *Pina-D1*. Consequently, it was argued that if a variety possessed either of the mutant alleles (*Pina-D1b* or *Pinb-D1b*) at these loci, the resultant grain was hard in texture. Although a number of other alleles have now been detected at these loci (Morris 2002), three genotypes predominate in released cultivars; “soft”, *Pina-D1a/Pinb-D1a*; “hard”, *Pina-D1a/Pinb-D1b* and “extra hard”, *Pina-D1b/Pinb-D1a* (Cane et al. 2004). The water absorption of these three genotypic classes is positively correlated with grain hardness. In the work of Cane et al. (2004), varieties with the “extra hard” genotype absorbed 3.5% more water than varieties with the “hard” genotype and 8.3% more than those with the “soft” genotype. However the distinction between the water absorption of the “extra hard” and “hard” classes

was not observed by Martin et al. (2001). Both of these studies showed a drop in milling yield associated with the "extra hard" class. Beyond this major gene for grain texture, numerous QTL associated with grain hardness have been reported.

Only the 5D genome of hexaploid wheat has the *Ha* locus and the homologous loci on chromosomes 5A and 5B are absent. Consistent with this situation in hexaploid wheat, the *Ha* locus is also missing from the tetraploid progenitor (AABB), although present in the diploid progenitors – a major deletion event is therefore assumed to have occurred after the polyploidization event that generated the AABB tetraploid wheat. The *Ha* locus is defined by three genes, grain softness protein (*Gsp*), puroindoline a (*Pina*) and puroindoline b (*Pinb*) and extensive sequence analyses on the region were carried out by Chantret et al. (2004, 2005). Based on genomic DNA sequences identifiable in tetraploid wheat, the 5' boundary of the *Ha* locus was defined by the *Gsp* gene that is also present in the A, B genomes of tetraploid wheat. The 3' boundary was defined by a gene cluster (called *Gene7* and *Gene8*) also present in A, B genomes of tetraploid wheat. The *Ha* locus can therefore be defined by a ca 55 kb segment of genomic DNA containing the *Pina*, *Pinb*, two degenerate copies of *Pinb*, *Gene 3* (present only in the D genomes) and *Gene 5*. *Gene 3* and *Gene 5* were not annotated by Chantret et al. (2005). The study by Chantret et al. (2005) indicated major differences between the D genome progenitor locus and the D genome locus in hexaploid wheat and these included the deletion of about 38 kb of DNA sequence in the hexaploid locus relative to the diploid locus.

The analysis of the barley *Ha* locus (Caldwell et al. 2004) has indicated that some gene clusters such as the gene cluster 2, GC2 (annotated as *VAMP*, *GlcNAc*, *Gsp*) are conserved between wheat and barley. Rearrangements have however occurred in the locus and the puroindoline equivalents in barley (hordindolines) are upstream from GC2 instead of downstream as in wheat and they are also in the opposite orientation. The interpretation of grain hardness in barley has not focused on milling attributes as has been the case for wheat but has instead focused on resistance of the grain to invasion by pests and diseases (Caldwell et al. 2004).

21.5 Traits that Are Not Analysed at the Genomic Level to Date

21.5.1 Milling Yield

Milling yield is the amount of flour that can be obtained from a given weight of grain. It reflects the amount of endosperm and the ease with which it can be separated from the germ and bran. The separation of endosperm as flour is considered to relate to the degree of cross-linking of cell wall components (arabinoxylans) and hence the intimacy of the bran-endosperm

interconnection. Milling yield is thus a measure of the actual amount of endosperm available, which is a very important component of the grain for commercial purposes (Simmonds 1989; Finney et al. 1987). Flour is extracted primarily from the endosperm of a wheat grain, and is composed of starch granules encased in a protein matrix. Surrounding the endosperm, the bran, along with the embryo (germ) forms the non-flour fraction of the grain. The quantity of flour able to be extracted from a grain is consequently a function of the bran to endosperm ratio, which is dictated by grain size and morphology, as well as the ease with which the endosperm is released from the bran (Simmonds 1989). Grain size and morphology characters are under both environmental and genetic control. A number of reports exist of genetic associations with milling yield and many have not been experimentally related to grain size and shape.

21.5.2 *Water Absorption*

The quantity of water absorbed by flour during dough formation varies between varieties. For bread products relatively high water absorption is required, whereas for biscuits and noodle production lower water absorption is desirable (Simmonds 1989). The water absorption of flour is heavily influenced by grain texture, grain protein content and the level of non-starch polysaccharides. Although the level of starch damage can be altered by the conditions used during milling, this attribute is related to grain texture and therefore under significant genetic control (as discussed in Section 21.4). Although non-starch polysaccharides have been shown to influence the water absorption properties of wheat flour (Shogren et al. 1987; Shewry and Morell 2001; Howitt et al. 2003), this has not yet been clearly demonstrated through genetic association studies.

21.5.3 *Grain Protein Content*

As protein is responsible for much of the functionality of flour and the quantity of protein within each grain forms a key quality criteria. The quantity of protein in a wheat kernel, expressed as a proportion of kernel weight, is heavily influenced by both the nitrogen and carbon supply to the developing grain. Consequently, a strong inverse relationship between grain yield and protein concentration exists (Cooper et al. 2001; Fabrizius et al. 1997). It would therefore be expected that many of the genes responsible for the grain yield and grain weight (Röder et al. 2007) of wheat would also influence grain protein content. However, genes that increase grain protein without reducing grain yield, would be of more interest to wheat breeders attempting to improve both grain yield and protein concentration simultaneously.

Protein content relates to the amount of gluten in a sample and is very important measure of rheological properties (Wall 1979; Payne et al. 1984). Different wheat products have their own specific protein content requirements. For examples, biscuit manufacture requires low protein content (<11%), while pasta manufacture requires a minimum of 12% protein content. Manufacturing YAN noodles needs at least 11% protein content, while WSN noodles requires 9–9.5% protein content (Simmonds 1989).

A gene influencing protein content, independent of grain yield, was identified in *Triticum turgidum* on chromosome 6BS (Joppa et al. 1997). This gene (*Gpc-B1*) was transferred to bread wheat, resulting in the variety “GluPro” (Khan et al. 2000). Since then, the gene has successfully been incorporated into commercial varieties such as “Lillian” (DePauw et al. 2005), and “Somerset” (Fox et al. 2006). The detailed analysis of the *Gpc-B1* gene (designated *TaNAM-B1* by Uauy et al. 2006; Distelfeld et al. 2007) indicates its fundamental role is to act as a transcription factor controlling the timing of leaf senescence and the remobilization of zinc, iron and manganese. The increase in grain protein appears to be a pleiotropic effect associated with increased remobilization of protein from the senescent leaves (Distelfeld et al. 2007).

Numerous reports (Breseghello et al. 2005; Prasad et al. 2003; Groos et al. 2004; Turner et al. 2004) have identified QTL associated with protein content, and that these are distributed across most of the genome. Unfortunately, many of these studies were performed without accounting for grain yield, so it is difficult to determine if selection for the high protein alleles within a breeding program would result in an increase *per se* in protein content without a corresponding drop in grain yield. However, it is interesting to note the large number of grain and flour protein content QTL that are coincident with grain yield and grain weight QTL identified in alternative populations. This tends to confirm the strong relationship between protein content and grain yield.

21.6 Impact of New Technologies

The impact of technologies that drive the high throughput analysis of protein and carbohydrate components of the grain and the genes that underpin variation in these components, will open up new requirements for integrating large and complex datasets. In wheat flour, for example, 1,300–1,500 polypeptides have been identified using 2D electrophoresis (Skylas et al. 2001; Gobaa et al. 2007) and these represent approximately 20–25% of the total genes expressed in wheat endosperm during development, based on sequence analyses of expressed sequence tags (ESTs) randomly cloned from mid-development endosperm tissue (Clarke et al. 2000). Since not all the genes expressed during development contribute to proteins in the mature kernel, it is evident that carrying out the appropriate proteomics analysis can identify a significant proportion of the genes contributing to protein components of wheat flour. The advantage of this

level of analysis, to complement the work ongoing using DNA probes, is that the effects of environment on protein accumulation during grain filling is also assessed as well as identifying the particular allele of the protein. Aspects of the allergic response of humans to wheat and barley products such as celiac disease have been reviewed (Kasarda 1994; van Heel and West 2006) and identifying key components responsible for these medical challenges is now becoming possible with the new analytical technologies.

Rapid advances in separation technologies such high performance liquid and gas chromatography linked to mass spectrometers can now define the metabolomics area of grain quality. Fast screens for vitamin A, iron and zinc in food products are possible in order to screen for genetic variants of grain that have higher levels of these compounds (HarvestPlus biofortification program; www.harvestplus.org). Similarly screening for low phytate levels is also important in the context of improving the nutritional attributes of grain because phytate interferes with the absorption of these and other minerals by the human gut.

The datasets derived from proteomics and metabolomics interface with the datasets from genome sequencing studies and microarray analyses of expressed genes in developing grain (Druka et al. 2006), germinating grain (Potokina et al. 2002, 2006) and plant responses to disease and abiotic stress (Walia et al. 2006), and web-based technologies form the basis for integrating these datasets. Assigning functions to specific genes is now also more efficient in cereals through the use of small RNAs or TILLING, to delete the activity of target genes through specifically removing messenger RNA or identifying mutations, respectively (Fu et al. 2007; Slade et al. 2005). A published example of a large-scale integration project is the Physiome project which is compiling all the information related to the human body (Hunter and Borg 2003) and this provides a good guide for dealing with specific areas such as grain quality evolving in-step with other changes in the food chain.

21.7 Conclusions

The quality attributes of cereal grains are valued in the context of a complex food chain that integrates outputs achievable by breeding, production, processing and consumer preferences. Each of these areas are constantly changing either in a controlled way (introduction of new technology) or in an uncontrolled way due to environmental variables and climate change, as well as changes in the market place resulting from advances in large-scale processing equipment and changes in consumer preferences. There is therefore a need for the quality features of wheat and barley grain to be tailored to keep step with these changes. The advances in the genomics of quality described in this chapter provide the basis for ensuring that the genetic approaches encompassing the complexities of the gene networks underpinning quality attributes can meet the challenges presented by the rapid changes occurring within the food chain.

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